THESIS

IDENTIFYING GRASS-LEGUME BICULTURES TO INCREASE ABOVE AND BELOWGROUND BIOMASS PRODUCTION AND IMPROVE TRADITIONAL FALLOWS IN CROP ROTATIONS OF THE ANDEAN HIGHLANDS

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Spring 2021

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ABSTRACT

IDENTIFYING GRASS-LEGUME BICULTURES TO INCREASE ABOVE AND BELOWGROUND BIOMASS PRODUCTION AND IMPROVE TRADITIONAL FALLOWS IN CROP ROTATIONS OF THE ANDEAN HIGHLANDS

In the high Andes of Peru, intensification of crop rotation and agricultural land-use is reducing the practice and duration of traditional fallow (based on natural establishment of native vegetation). These fallows represent one of the main traditional soil management practices to sustain long-term productivity, while also providing key forage resources in these mixed crop-livestock systems. Improved forage-based fallows, with the intentional seeding of more productive annual and perennial forages, offer great potential for producing forage and contributing to soil restoration under intensified contexts; however, there remains a gap in knowledge about which plant species can best optimize tradeoffs between forage production and belowground inputs to support long-term soil fertility and contribute to the multifunctionality of Andean agroecosystems. To address this issue, a pot study was conducted with two contrasting soils to evaluate the above and belowground productivity of all possible grass-legume pairs involving five grasses (oat (*Avena sativa*), ryegrass (*Lolium multiflorum*), festulolium (*Lolium x Festuca genera*), brome grass (*Bromus catharticus*), *and* orchardgrass (*Dactylis glomerata*), and four legumes (vetch (*Vicia dasycarpa*), red clover (*Trifolium pratense*), black medic (*Medicago lupulina*), *and* alfalfa (*Medicago sativa*)) in comparison to the performance of each species in monoculture. Grass-legume bicultures resulted in significant overyielding, producing 65% and 28% more total dry biomass and total N uptake on average than species in monoculture, respectively. Grass-legume shoot biomass production yielded 67% more compared to monocultures, while root biomass was on average 58% higher in bicultures than in monocultures. For aboveground biomass, production differences between grass-legume bicultures were significantly influenced by the species of legume present, while belowground biomass

was more affected by the grass species present in the bicultures. Roughly 80% of the mixtures achieved a mean land equivalent ratio (LER) > 1.0. When examining total biomass production, the most successful bicultures were oat-vetch (LER=1.87), vetch-festulolium (LER=2.31), vetch-orchardgrass (LER=1.87), oat-red clover (LER=1.62), and red clover-ryegrass (LER=1.46). When examining partial LERs (the component of the LER attributed to each species), we found that overyielding in bicultures was mainly driven by increases in the biomass of the component grass species. Our findings suggest that mixtures of key functional species (e.g. grass and legume, annual and perennial species) offer greater promise in improved fallows compared to monocultures of the respective species. Additionally, I suggest that strategically designed improved fallow mixtures, with emphasis on perennial species that support long-term root inputs, can best support soil health and the multifunctionality of Andean agroecosystems.

ACKNOWLEDGEMENTS

Firstly, I would like to thank my advisor Steven Fonte, for all his support, and invaluable mentorship that he has given me over the past two years. I would like to express my gratitude to my co-mentor, Dr. Steven Vanek, for all his suggestions and guidance during my studies at CSU. I also appreciate the contribution of my committee members, Dr. Jessica G. Davis and Dr. Meagan Schipanski.

I would like to acknowledge the McKnight Foundation for supporting the research project "Expanding the impact of improved fallows and landscape management for soil fertility and ecosystem services". This project is implemented and led by Grupo Yanapai (Peruvian NGO), and through them I would like to thank my Peruvian colleagues, students from Universidad del Centro del Peru and Universidad Nacional de Huancavelica for their participation in data collection, and the Andean communities and farmers for their inspiration and collaboration with the development of this project.

Finally, I would like to thank my family for his motivation and Sarah Fonte for all her support during my time in the USA.

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INTRODUCTION

Traditional fallows represent an ancestral soil management strategy to regenerate soils in many smallholder cropping systems around the world. In the high Andes of Peru, these fallows generally consist of periods for re-establishing native vegetation cover (mainly grasses, forbs and shrubs) within an extended crop rotation. The fallow portion of the rotation typically lasts from 1 to 15 years, largely depending on altitude. These fallows play multiple agroecological roles. They provide a grazing resource for livestock and organic inputs in the form of plant residues and manure that contribute to soil organic matter (SOM) and nutrient pools and the long-term productivity of soils (Pestalozzi, 2000; Sarmiento & Bottner, 2002). At the same time, by maintaining soil cover these fallows support soil conservation and improve water dynamics in these mountainous landscapes (Sarmiento, 2000). Despite the benefits of traditional fallows, changes in land use and the intensification of crop rotations, due to population pressures and agriculture modernization, are leading to fallows becoming shorter or disappearing entirely in some highland areas (Fonte et al., 2012). This trend is especially notable in agricultural communities located near urban areas and large agricultural markets. The shortening of fallow periods not only diminishes already scarce forage resources in these mixed crop-livestock systems, but also threatens the long-term maintenance of soil fertility via manure inputs and the positive effects of perennial fallow vegetation (King & Blesh, 2018). This situation suggests the need for targeted innovations to support soil health and forage needs.

To address these concerns, the concept of agroecological intensification provides a framework for developing alternative farming systems that can increase productivity while supporting the multifunctionality of the agroecosystems (Wezel et al., 2015). The idea of "improved fallows" refers to the introduction of cultivated plant species in the fallow stage of a crop rotation and has been proposed as a means to accelerate the recovery of soil fertility and other key agroecosystem functions (Sanchez, 1999). Improved fallows often include well-adapted, fast growing species to rapidly cover soils and

protect against erosion as well as legumes to support N accumulation for building SOM and overall soil fertility, but can also include species that contribute much needed fuel or fodder during the fallow stage. This strategy has been tested widely in the tropics of Africa and Asia (Nyamadzawo et al., 2008; Qingjie et al., 2009) and fits well within the framework of agroecological intensification, but has received less attention in other smallholder environments such as the Andean highlands. In the tropical Andes of Venezuela, the intentional seeding of native legumes (e.g., *Lupinus meridanus*) has been suggested as a promising strategy to build soil N pools and address shortening fallow periods (Sarmiento et al., 2012). Similarly, in the Peruvian highlands planting perennial grass-legume mixtures has showed to enhance regeneration of soil nutrient pools and soil physical conditions (Rolando et al., 2018). Also working in Peru, (Vanek et al., 2020) tested different improved fallows options together with farmers and found that improved fallows, especially those with alfalfa (*Medicago sativa*), supported potato productivity and quality, while also providing additional forage for livestock needs. Improved fallows thus offer a promising multifunctional strategy, connecting higher productivity to soil health. However, there are gaps in knowledge about strategic plant species associations for improved fallows that can optimize aboveground resources (i.e., forage) and belowground organic inputs for soil fertility restoration to achieve sustainable intensification of high Andean agroecosystems.

Plant diversity is typically high in traditional Andean fallows (e.g., De Valença et al., 2017), and it is likely important to maintain some level of functional diversity within improved fallows and similar agroecological approaches (e.g., cover cropping). This diversity helps ensure the multifunctionality (capacity of ecosystems to provide multiple ecosystem functions and services at the same time (Manning et al., 2018)), resilience (socio-ecological capacity for adaptation and transformation (Cabel & Oelofse, 2012)), and productivity of Andean cropping systems. Carefully selecting species for mixed (i.e., intercropping) systems, such as associations of legume and non-legumes, can foster synergistic interactions to increase biomass and harvest productivity (Vandermeer, 1989). Supporting this idea, numerous studies in natural, grassland, and agriculture systems have explored the effect of plant diversity

on plant productivity and other ecosystem services that these species can provide. For instance, Tilman et al., (2014) reviewed long and short-term biodiversity research across Europe and North America and found that biodiversity effects on plant productivity and ecosystem function are mostly due to complementarity between plant species, or the more effective use of the resources due to different species (or functional groups) filling different niches in the ecosystem (Kahmen et al., 2006). Interspecific facilitation effects can also occur, where one plant species creates favorable conditions for another, mediated by nutrient supply, physical support, pest/predator relationships, or other mechanisms (Brooker et al., 2015). Both complementarity and facilitation can act to increase water use efficiency and the total nutrient supply available to plant associations, thus supporting greater productivity. Others have shown that having multiple plant functional groups can increase overall productivity, but also provide key agroecological benefits (e.g., weed and pest suppression, increasing plant residues, and supporting pollinator activities) enhancing the overall functionality of agroecosystems (Blesh, 2017; Isbell et al., 2017).

These positive interactive effects of functional species mixtures also have implications for soil functioning. For example, legumes fix N, which can then be transferred to non-legume species allowing for an increase in the total N cycling, a key limitation in many agriculture systems (Schipanski & Drinkwater, 2012). Similarly, mixtures of fast-growing species such as annual grasses and legumes can deliver early benefits when N fixed by legumes supports the establishment of slower-growing mixture components that contribute to long-term soil and agroecosystem functioning (Wick et al., 2017).

Root growth and interactions play an important role in determining mixture performance and their potential to benefit SOM and nutrient pools. For example, perennial mixtures with legumes allocated substantially more C and N belowground than their respective monocultures, mainly due to an increase of root biomass accumulation throughout the soil profile (Fornara & Tilman, 2008)**.** Increased plant diversity can also support belowground biodiversity and activity in the soil, with implications for multiple soil functions (Marshall et al., 2016). Thus, many authors have suggested that roots and associated

rhizodeposits represent key organic inputs that contribute disproportionately the building of SOM and multiple ecological functions that help to restore soil health and productivity (Rasse et al., 2005; Schmidt et al., 2011)**.**

In spite of great potential of highly diverse plant species mixtures to support multiple ecosystem functions, such mixtures are not always practical and similar benefits can often be achieved with simple associations of just two or three species from different functional groups (DeHaan et al., 2010). In addition, species interactions are likely environment specific and more information is needed to determine the optimal species combinations in high Andean contexts. Building on the potential for species mixtures to support key ecosystem services, and as part of a wider effort to develop forage-based fallows in the Peruvian highlands that can maintain soil health and address critical shortages in forage (Vanek et al., 2020)**,** this study examined the following important questions: first, to what extent do species mixtures (or bicultures) promote above and belowground biomass production relative to monocultures across differing soil conditions? Second, are there specific bicultures (or component species) that are especially promising for supporting aboveground vs. belowground biomass production, and associated implications for forage vs. soil improvement use? We hypothesized that bicultures would generally perform better (including root biomass production and whole plant N uptake) than monocultures. Additionally, promising species bicultures will respond consistently across soils with contrasting fertility levels. To address these questions, we used a pot study to evaluate the above- and belowground production of forage bicultures vs. monocultures within two soils from the central Peruvian Andes. We evaluated grass-legume species combinations between four legumes and five grass species known to be relatively well-adapted to Andean conditions and identified as promising options for improved fallows.

MATERIAL AND METHODS

Study site and soil collection

The pot experiment was carried out in a protected outdoor location in the district of Quilcas, located in the Junín region of Peru (11°55'21.50 "S, 75°14'50.56 "W). At an altitude of 3,506 m, with an average monthly temperatures of 14°C, and average rainfall is 700 mm yr^{-1}, occurring mainly in the growing season from October through April (García, 2011).

In mid-November of 2014, soils were collected from farmers' fields in two agricultural communities of the central Andean region. The first soil was collected from a field adjacent to the study site in the district of Quilcas, while the other soil came from the community of Castillapata (12°44'2,61 "S, 74°48'56,18 "W) located in the Huancavelica Region, at an altitude of 3,930 m. This site has an average monthly temperature of 9 °C and annual precipitation of ~ 703 mm yr^{-1}, again with most rainfall occurring from October to April. Both sites are characterized by rugged topography and high susceptibility to erosion and largely dominated by Inceptisols (USDA soil taxonomy), with pH conditions ranging from acidic to very acidic. The soils collected from Quilcas have low to moderate SOM, with a clay loam texture class, while the lower temperatures in Castillapata result in greater accumulation of SOM, and with sandy loam type soil (Table 1).

Both soils came from fields in the first year of traditional fallow, after a 3-5-year crop sequence. These rotations typically involve potato (*Solanum tuberosum*), Andean tubers (e.g., *Oxalis tuberosa*), legume (e.g., *Vicia faba*), corn (*Zea mays*), and forages (*Lolium multiflorum and Trifolium pratense*) followed by a 2-3-year traditional fallow at the Quilcas site, while in Castillapata the crop rotation usually includes potato (*Solanum tuberosum and S. andigena*), and forages (barley (*Hordeum vulgare*) or oats (*Avena sativa*)) followed by a 3-7 year fallow, which tends to increase with altitude due to decreasing temperatures and associated productivity of the fallow vegetation.

Experimental design and establishment

Soils from each site were collected to a depth of 20 cm and passed through a 5 mm sieve to remove rocks and large organic debris. Soils were thoroughly mixed to homogenize material separately for each site. A representative sub-sample (~2 kg) of each soil was taken, air-dried, passed through a 2 mm sieve, and sent to the Soil and Plant Nutrition Lab at La Molina University (Lima, Peru) for analysis of physical and chemical properties (Table 1). For establishment of the pot study, soils were mixed with sand (3:1 soil:sand ratio by volume) in order to ensure drainage. Pots were constructed out of 10 cm diameter PVC pipe cut into 40 cm sections, with a cap affixed to the bottom. Each pot was filled approximately 3 kg of the soil-sand mixture, with six drainage holes at the base (~ 3 mm in diameter). In order to help regulate temperature and humidity, the pots were placed in four separate trenches (~5 m long, with rock layer at the bottom for drainage), and the trenches were backfilled, such that soil in each pot was level with the surrounding ground.

Forage species from two plant functional groups (legumes and grasses) were selected based on consultation with local farmers and a preliminary pot trial, which evaluated the productivity of approximately 60 different species (or varieties) of cultivated and native plant species under conditions similar to this study (data not presented here). A total of five grasses, including one annual species (oats) and four perennials, were selected: oats (*Avena sativa* cv. Mantaro 15M; OATS), ryegrass (*Lolium multiflorum* cv. Tama; RYEG), Festulolium (*Lolium* x *Festuca genera*; FELO), brome grass (*Bromus catharticus*; BROM), and orchardgrass (*Dactylis glomerata*; ORCH). Four legumes, including one annual (vetch) and three perennials were selected: vetch (*Vicia dasycarpa*; VETCH), red clover (*Trifolium pratense*; REDC), black medic (*Medicago lupulina*; MELU), and alfalfa (*Medicago sativa;* cv. Ranger; ALFA). In December 2014, treatments were established using all possible pair-wise combinations of the two functional groups (grass-legume) as well as each species planted in monoculture. These 29 treatments were planted within both soil types resulting in a total of 58 treatments. Each plant treatment and soil combination were present in four replicate blocks in a randomized complete block design, resulting in a

total of 232 experimental units. The density of seed planted in each pot was selected based on the seed size and anticipated growth rate of each species allowing plants to fill the area of the pots to a similar degree. Monocultures were always seeded at double the rate of individual species in bicultures (Table 2). Seeds were sown into the pots at slightly higher densities than needed, covered with a thin layer of sand, and treatments were thinned to the desired densities after two weeks. In case of poor germination, a separate set of seeds were pre-germinated with gibberellic acid (1 ml L^{-1}) and maintained in a moist paper towel at ~25 °C [1] to break dormancy. These seedlings were used as needed in the first few weeks of the experiment to achieve desired densities in several pots with missing plants. Each block was covered with a removable plastic cover on a wooden frame to protect against hail damage during severe storms. The experiment was watered weekly when there was insufficient rainfall. Pots received no fertilizer or pesticide inputs and were weeded regularly to limit the growth of undesired species.

Plant harvest and data collection

Pots in all treatments were harvested in April 2015 (end of the rainy season and 141 days after sowing), when 75% of the vetch reached the flowering stage, based on typical timing for fodder harvest in the region. At harvest, pots were destructively sampled to assess above and belowground biomass production and total N content. Plants and soil were removed from the pots and deposited onto a tray. Soil was removed from plant roots by gently breaking apart large aggregates, and the plants were sorted by functional group. Roots were then separated from the aboveground portion of each plant (stems, leaves and flowers) using scissors and rinsed with water on top of three sieves of sizes 2 mm, 1 mm, 0.5 mm to capture the roots that were broken during root washing. All plant components were placed in separate paper bags, dried at 60 °C, and weighed. To determine N content of the biomass, a subsample of the dried plant components from each pot were combined in their relative proportions (according to the biomass of each component) for a single composite sample (> 250 mg). These subsamples were then ground and sent to the Soil and Plant Nutrition Lab at La Molina University for analysis of total N using the Micro-Kjeldahl Method.

Calculating the Land Equivalent Ratio

Total dry biomass of the bicultures and monocultures were used to calculate the Land Equivalent Ratio (LER) for each biculture. LER is defined as the relative area of land required to produce the same amount of biomass in monoculture as that produced when growing the plants in a mixture. An LER >1 indicates that polycultures are more productive than their respective monocultures (Vandermeer, 1989). The LER was calculated using the equation:

$$LER = (P_L/M_L) + (P_G/M_G) \quad (1)$$

Where P_L and P_G are the dry biomass for the legume and grass, respectively, under polyculture conditions and M_L and M_G represent the biomass of the legume and grass, respectively, when grown as monocrop. For this calculation M_L and M_G were represented by a pooled average of yield across all replicates of the component monoculture, while P_L and P_G where based on individual replicates of each treatment, so that one LER result was calculated for each experimental unit. We also considered the partial LERs for each treatment, (P_L/M_L) or (P_G/M_G), which assessed the relative contribution of each species to any observed overyielding.

Statistical approach

To examine plant treatment and soil effects on total plant biomass, root biomass, shoot biomass, and total plant N content (roots + shoots) we used two-way ANOVA with plant treatments (29 levels) and soil type (2 levels), and their interaction considered as fixed effects and block (or replication) considered as a random effect. Due to the strong interest in developing perennial forage mixtures and the relatively high productivity of the annual species (oats and vetch), the above analyses were also conducted using only the treatments with perennial species. Additionally, contrasts were used to compare plant species bicultures vs. monocultures, for both sets of analyses, with and without annuals.

A second model was used to compare plant bicultures using LER as the main response variable, with LER calculated separately for total biomass as well as root and shoot biomass. This model

considered grass species (5 levels), legume species (4 levels) and soil type (2 levels) and all possible two and three-way interactions as fixed effects, while block was considered as a random effect. Similar to the two-way ANOVA mentioned above, the analyses were conducted both with and without annual species.

For all analyses, estimated means were compared using Tukey tests. In addition, 95% confidence intervals drawn from the Tukey test were used to test if LERs were significantly different from 1. Meanwhile, a Bonferroni correction was used for specific comparisons, such as statistical contrasts between biculture vs. monocultures. Square root transformation was applied to all response variables except for several variables (total biomass, shoot biomass, root LER), where natural log transformations were used to meet ANOVA assumptions (variance homogeneity and normality). ANOVA results were considered as statistically significant at $\alpha=0.05$. All analyses were carried out with R statistical software (R Core Team 2019), using the lme4 (Bates et al., 2015), lmerTest (Kuznetsova et al., 2017)**,** and emmeans (Lenth et al., 2019) packages.

RESULTS

Plant treatment and soil effects on biomass production and N uptake

Overall, 92% of pots maintained the desired treatments and were harvested as planned. There was a small amount of loss of experimental units in the trial, where one or both of the component species died before harvest. These pots were not from any particular treatment and were excluded from the analysis (Table 2).

Total plant biomass, aboveground biomass, root biomass, and total N uptake were influenced by both treatment and soil type ($p < 0.001$; Tables 3 and 4). Plant treatments that included the annual oat and vetch species resulted in the highest productivity based on total biomass, while the lowest biomass across all treatments corresponded to the perennial legume monocultures (Table 3). For soils, total plant biomass was nearly 2.5 times higher in the Quilcas soil, than in soil from Castillapata, while the general lack of significant interactions between soil and plant treatments showed that differences in plant treatment were largely consistent across soil types. One exception is noted for root biomass (interaction $p = 0.018$); in this case, although most treatments produced more root biomass in the Quilcas soil, there were some treatments, such as festulolium-vetch, brome-vetch, and vetch monoculture, that had similar root biomass across the two soils (Table 4). Perennial treatments with high productivity included ryegrass and festulolium, either in biculture or monoculture (Table 3).

Statistical contrasts comparing biculture and monoculture, in terms of biomass and N uptake averaged across the two soils, indicated that bicultures had 65% higher total biomass and 28% greater total N uptake than monocultures ($p < 0.001$; Fig. 2). Consideration of only the treatments with perennial species showed that the bicultures, on average, yielded 36% and 13% more than monocultures in terms of total biomass and total N uptake, respectively ($p < 0.001$; Fig. 3).

Comparing LER across mixtures

When looking at total biomass production across the grass-legume bicultures, vetch paired with festulolium, orchardgrass, oat, brome, and ryegrass as well as red clover-oat, demonstrated overyielding with LER values significantly greater than 1 ($p < 0.05$; Fig 4). While mean LERs for many other treatments were greater than 1 (Fig 4.), high variability in many treatments led to the inclusion of the critical value (LER = 1) within their 95% confidence intervals for biomass, thus indicating less confidence in their ability to consistently overyield.

The LER for total biomass was influenced by the type of legume in the mixture ($p = 0.001$, Table 5), such that mixtures including vetch had an average LER for total biomass of 1.87, whereas average LER values for red clover (1.25) and alfalfa (1.14) were lower. When examining LER values for above- and belowground plant components separately, the aboveground biomass followed the same trend as total biomass, such that LER values were significantly influenced by the type of legume ($p < 0.001$, Table 5), with no significant interactions with other factors. Whereas, variation in LER values corresponding to belowground biomass was influenced by the grass species present ($p = 0.049$, Table 5). This is largely explained by mixtures with oats having higher LER values for root biomass, when averaged across soils.

When only considering treatments with perennial species, LER values for total biomass and belowground did not show significant differences between treatments (Table 5). LER values for aboveground biomass were significantly influenced by the type of legume present ($p = 0.028$, Table 5), such that treatments with black medic in association with any grass seem to have higher LER. However, it should be noted that black medic had relatively low performance in monoculture (Fig. 1, Tables 3 & 4).

Partial LERs (calculated as the term corresponding to each species in the summation defining LER, see equation 1 in methods) were examined to understand how each species behaved in biculture. Partial LERs showed that although legume type present explains differences in LER across the various bicultures (Table 5), the grass component of each biculture appears to be the main driver for higher or

lower LER (Fig. 4). On average, partial LER for the grass component across all bicultures was 0.96, while the partial LER for legumes averaged 0.43. Among the most productive bicultures, vetch-oat, vetch-ryegrass, and red clover-oat all had high aboveground LER values (Fig. 5). The grasses within these bicultures had partial LERs of 1.20, 1.43, and 1.39, meaning that with just half of the density of plants that were present in the monocultures, the bicultures produced more biomass. At the same time, the legume component in these bicultures achieved 0.60, 0.30, and 0.25 partial LER, respectively. In the case of vetch-festulolium, vetch-orchardgrass, and vetch-brome bicultures, grasses had partial LERs 1.61, 0.80 and 0.53, respectively, while vetch in these bicultures had partial LER values of 0.94, 0.97, and 1.28, respectively (Fig. 5).

When examining partial LERs for belowground biomass, grass roots largely explained variation in LER between treatments. Vetch-oat, vetch-orchardgrass, black medic-oat, red clover-oat, and vetch-festulolium had the highest LER for roots. When oat was present in these mixtures it had generally high partial LER values (on average 1.6), no matter what legume it was paired with (Fig. 5). Legume appeared to influence the partial LER of grasses; for instance, black medic led to overall lower partial LERs for grasses (Fig. 5B), in comparison to red clover and alfalfa (Fig. 5C, D).

DISCUSSION

This study tested a range of forage mixtures of annual and perennial legumes and grasses and how these compare with the performance of their respective monocultures under Andean environmental conditions. Our results broadly support our hypothesis that plant mixtures can enhance above- and belowground biomass production relative to monocultures, with important implications for multiple ecosystem services, such as C sequestration, erosion control, soil fertility restoration, and forage production.

Overall performance of bicultures vs. monocultures

Our findings indicate that combining grass and legume forage species increased total biomass production relative to the same species grown in monoculture (Fig. 2 & 3; Table 3). Average LER values of ~1.4, suggest that ~40% more area would be needed to produce the same amount of biomass under monoculture, on average, than with the two-species mixtures tested here. The LER achieved in our study is in line with the average LER of 1.22 reported in a meta-analysis by Yu et al., (2015) that compared 189 experiments across five continents, with roughly 85% of the studies looking specifically at intercropping systems based on grass-legume mixtures. In our study, trends for aboveground biomass were similar to results for total biomass, where bicultures overyielded by 67% compared to their respective monocultures (Fig. 2B). This result is consistent with other studies from grassland systems, where more diverse species mixtures have been shown to exceed the average biomass yield of their component monocultures by 70% to 200% (Cardinale et al., 2007; Tilman et al., 2014). Furthermore, our results suggest that the mixture benefits from these tested species are similar across two soils with widely differing productivity, indicating that these sorts of mixtures may be applicable across a range of different soil types in Andean agricultural landscapes.

In agricultural systems, the beneficial effects of plant diversification on productivity are not simply due to increased species diversity *per se*, but more often to interactions between key functional groups that allow mixtures to be more productive (Ren et al., 2014)**.** An example of this sort of functional interaction has been reported previously for legumes growing with other plant functional groups, especially grasses **(**Fornara & Tilman, 2008**;** Hooper & Dukes, 2004**;** Faucon et al., 2017**)**. Including legumes in mixtures often leads to increased N uptake and total biomass production relative to monocultures (Sainju et al., 2005). This same trend was observed in our study, where grass-legume mixtures had 28% greater N in their biomass than their respective monocultures, and this was associated with 65% higher total biomass production compared to monocultures (Fig. 2A & D). This phenomenon can be due to a variety of mechanisms; however, we suspect that enhanced N cycling due to facilitation of N fixation is at least partly responsible for the trends observed here. Others have shown that grasses can effectively compete for available soil N, thus forcing legumes to meet their N demand through N fixation (Nyfeler et al., 2011). This increases the overall input of N into the soil-plant system, and the legume N then eventually becomes available to the grasses through variety of different means (e.g., decaying of senescent roots/nodules, release in exudates, mycorrhizal mediated transfer), thus enhancing the productivity of the system. In one example from the tropics, (Eaglesham et al., 1981) reported that maize N uptake increased by roughly 90% when grown in association with cowpea compared to maize grown in monoculture; they also used ^{15}N isotopes to show that most of the N increase was from atmospherically-derived N fixed by the cowpea at their physiological maturity (~85 days after seeding). In this study, such facilitation of N is evidenced by the positive response of grasses to the biculture conditions, with an average partial LER 0.96 (where 0.5 indicates no change between mixture and monoculture), whereas legumes on average had a partial LER of 0.48. The increase in total N uptake in these unfertilized soils and the fact that legumes did not perform as well in biculture suggest that the legumes were able to fix additional N, but that most of this N may have been utilized by the grasses.

Interspecies complementarity exists as another possible mechanism to explain the general increase in biomass and N uptake in mixtures vs. monocultures. Along with the observed effects on overall productivity, root biomass of the bicultures yielded 58% more than monocultures (Fig. 3C; Table 3). This general increase in root biomass suggests that mixtures are more effectively exploiting soil resources in the pots and provides evidence of complementarity. Our results are consistent with a global metanalysis that includes results from multiple experimental set-ups (e.g., small plots, greenhouse), where they found that plant mixtures, on average, produced ~29% more fine root biomass and ~45% higher total root annual biomass than plants grown in monoculture (Ma & Chen, 2016)**.** This result may be due to differences in root architecture or other important root traits that allow for improved resource extraction (Mueller et al., 2013). Beyond supporting improved resource acquisition and overall plant productivity, enhanced root growth under mixtures likely increases the direct deposition of root residues in the soil, which has important implications for soil C storage and SOM building (Rasse et al., 2005) and a range of other important ecosystem functions.

Comparing performance of different bicultures

Along with considering the general effects of mixtures vs. monocultures on biomass and N uptake, it is important to examine the differences between mixtures and the driving effects of different component species so that we can select for optimal performance of fallows. For example, among the twenty grass-legume treatments studied, the mixture with two annual species (i.e., oat-vetch) resulted in the highest above and belowground LER across sites (Fig. 2). Oat-vetch mixtures are widely known for forage production and quality across a range of agroecosystems and have been used as an agroecological option similar to the improved fallows of interest here, mainly as short-term cover crops with fallow-type benefits (Dhima et al., 2007; Finney & Kaye, 2017). We suspect that the greater oat-vetch performance observed here largely corresponds to their faster growth rate common among annual species. This trait likely translated into an ability to take up nutrients and rapidly invest energy to increase unit leaf area and biomass shortly after emergence and during early growth stages. In contrast, perennial grasses (e.g.,

Dactylis glomerata, Bromus sp.) tend to invest more energy to form multiple leaf sheaths in preparation for growth over a longer life cycle (Garnier, 1992). Others have suggested that the success of vetch-oat mixtures is at least in part due to high morphological plasticity of both species at different stages of mixture growth (Sheng et al., 2011)**,** or to improved light dynamics, where oat supports the vetch canopy increasing light interception (Mariotti et al., 2009). While the oat-vetch mixture may seem promising, it is important to note that this finding is likely an artifact of the relatively short duration of this study, and that over time perennial species would be expected to catch up and provide cover, C inputs, and forage for multiple years, and likely exceed the lifetime productivity of the annuals. Supporting this notion, findings of Vanek et al., (2020) indicated that oat-vetch mixtures in managed fallows in the central Andes, may be associated with greater soil C decline relative to perennial mixtures after 3 years of forage-based fallow. They attributed this to reduced root growth and residue return, since mixtures were only planted in the first year of a three-year fallow and plots with vetch and oat mixtures had low productivity in years 2 and 3 of the fallow. This strategy may thus need to be improved with the integration/association of perennial species, seeded together with annuals, to maintain growth beyond the first year.

Faced with the need for multi-year improved fallows that can protect the soil surface following the cropping phase of a rotation and beyond, the association of annual and perennial species offers a promising strategy to support high productivity in the first year, with continued above- and belowground biomass production in subsequent years (Wick et al., 2017). This is further supported by the perspective that perennial species allocate more to belowground biomass production than annuals, and provide a constant input of organic matter over time, thus contributing to soil restoration and the multifunctionality of fallows (Franzluebbers, 2015; King & Blesh, 2018)**.** However, such a mixture would depend on the ability of perennials to compete and fully establish in the first year. While we did not consider more complex mixtures that include both annual and perennial grasses and legumes in this study, our results suggest that the perennial species studied here can effectively compete, and in some cases thrive, in the presence of annuals. This was especially true for perennial grasses growing in mixtures with vetch. For example, we note that all perennial grasses combined with vetch had LERs greater than 1 (Fig 5A; Table

4). High partial LERs (i.e., values > 0.5) were observed for festulolium (1.61), ryegrass (1.43), and orchardgrass (0.80; Fig. 5A). At the same time, we note that perennial legumes grown in combination with oats were less successful and all displayed partial LERs that were less than 0.5, indicating that they were not able to effectively compete with the annual grass (Fig. 5B, C & D). However, the fact that these perennial legumes were in most cases able to establish and survive in the presence of a fast growing annual grass indicates promise, since these slower-growing legumes would be released from the intense competition in the second year of growth, once the oats are harvested and die.

Given the goal of optimizing the long-term performance of fallows, it is important to consider perennial mixtures that will survive beyond the first year after establishment. The treatments with ryegrass-red clover, festulolium-alfalfa, and black medic-brome all displayed relatively high LER values and were consistently supported by the relatively higher biomass of the grass species. However, it should be noted that brome-black medic, and festulolium-alfalfa had greater variability within treatments (i.e., large confidence intervals) and, thus, may have less reliable performance (Fig. 4). Also, brome-black medic, which appears quite promising based on LER (Fig. 4), had quite low total monoculture biomass of black medic (0.74 g pot^{-1}; Fig. 1; Table 3), thus inflating the LER value and bringing into question the viability of this mixture. Meanwhile, the ryegrass-red clover mixture achieved one of the highest LERs for a perennial mixture (Fig. 4) and had relatively high overall biomass (Fig. 1); further this mixture achieved the highest N content across perennial treatments (0.17 g pot^{-1}). This result suggests that even though red clover seemed to not perform well by itself (Fig. 5C), it substantially promoted grass productivity in mixture. Previous work on this mixture by (Dahlin & Stenberg, 2010) showed that ~44% of the ryegrass total N corresponded to the N transferred from red clover, which tended to increase in a low cutting forage regime compared to frequent harvesting practice. Perennial mixtures, including red clover-ryegrass, have also been shown to contribute to soil nutrient pools and support overall crop rotation productivity (Entz et al., 2002; Thilakarathna et al., 2016; Yuan et al., 2016), while contributing considerably to forage provision. This mixture not only supports productivity, but also the provision of

several ecosystem services in agricultural systems such as soil C storage, N supply, erosion control, and weed suppression (Schipanski et al., 2014). Improved fallow with the optimal mixtures of perennial species may be a way of reconciling current agricultural systems with the ecological benefits provided by traditional practices. However, this needs to be assessed beyond its first stage of growth considered in this study to better understand their ecological contribution over the course of a multi-year fallow.

While the artificial conditions used in this study (i.e., pots) limit our ability to generalize about forage/fallow performance at the field scale, our approach allowed us to assess species-specific root biomass production for species planted under mixed and monoculture conditions. This study provides evidence that root biomass did not depend on the type of legumes present in the biculture as was the case for aboveground biomass. For example, for the oat-vetch, festulolium-vetch, orchardgrass-vetch, oat-red clover, and ryegrass-red clover (Fig. 2), the actual belowground biomass of grass components were found to be higher than the corresponding monoculture root biomass. We suspect that this may be associated with the complementarity and facilitation mechanisms discussed above, where interspecific root interactions allow the grass roots to grow better and more fully explore the soil volume for resources (L. Li et al., 2013). For instance, others have shown barley roots to grow deeper and have enhanced lateral root development when paired with pea (Hauggaard-Nielsen & Jensen, 2001), and in maize-faba bean mixture, maize roots tend to be found under legume roots, increasing root length density across the soil profile (L. Li et al., 2006) in comparison to monocultures. Generally, we suspect that grasses performed better because of their root architecture; for example, oats, orchardgrass, and festulolium have a dense fibrous root systems that might offer a competitive advantage for proliferation of roots in the soil profile compared to tap-rooted species (e.g., legumes). Further, the interaction and entanglement of root can facilitated the optimization of resource access in mixtures (MacLeod et al., 2013; Marshall et al., 2016), including grass and legume species used in our study like *Lolium perenne* and *Trifolium pratense*. Our findings along with those from other studies suggest that grasses have intrinsic characteristics that give them greater plasticity in interactions with specific legumes (i.e., capacity of N fixation) giving better

possibilities for space and resource use, which can allow higher growth rates and subsequently support higher yields. However, this work requires further exploration under field conditions to understand what are the suitable mixtures and mechanisms that contribute to root growth in the Andes and other agroecosystems systems generally.

Implications for smallholder agriculture systems

We evaluated a wide range of grass-legume forage mixtures based on their LER and actual productivity. This work included testing of species that are currently managed and others that are not yet (e.g., *Festulolium, and Vicia dasycarpa)* well integrated within the complex and fragile Andean agricultural systems. Our findings indicate a simultaneous increase in above and belowground biomass in many mixtures. Previous studies have noted the significant association between high above and belowground productivity and the provision of multiple ecosystem services (L. Li et al., 2013; Mueller et al., 2013). As noted earlier, increased productivity in plant species mixtures has been associated with C sequestration, improved weed suppression, and reduced nutrient loss among others (Schipanski et al., 2014). This perspective reinforces our purpose for designing multi-functional fallow systems based on planted forages that might be optimized to include more than two functional species in the mixture (Sanderson et al., 2004; Vanek et al., 2020).

Our results suggest that a successful forage-based fallow in the Andes should integrate annual and perennial species chosen for maximum benefits between grasses and legumes. This will fit well with the timeframe of the traditional fallow in the Andes, which ranges from 1 to 4 years in the more intensive agricultural zones. In the first stage of the fallow period, vetch and oat may play a significant role, covering the soil and providing early growth, which would result in protection against soil erosion in the often steep and highly vulnerable soils of the Andean region. Vetch is a good forage legume that offers considerable promise for improving the productivity of perennial grasses including festulolium, ryegrass, or orchardgrass, suggesting that any of the perennial grass species could be added to the annual mixture. In addition, the integration of perennial legumes (e.g., red clover, black medic) may play a key role to

support years 2 through 4 of the fallow periods, and the productivity of upcoming crop rotations. This complex association can ensure the benefits of N fixation partly supporting greater residue inputs, which in a broader scenario may suggest a replacement or reduction of inorganic inputs (Yan et al., 2020). We are aware that more complex mixtures pose a challenge for farmers due to the complexity of planting and managing multiple species and the cost of seed and labor. However, our results suggest that the combination of perennial and annual, and legumes and grass species will deliver a desired above and belowground productivity and quality, meeting farmer expectations and supporting environmental resilience.

Our finding that grass-legume mixtures can enhance root production more than monocultures is significant. The importance of higher root biomass lies with the ability of roots to contribute more to C stabilization than aboveground residues and implications for the restoration of SOM (Rasse et al., 2005). Increasing SOM through root accumulation might positively affect soil health by improving soil physical conditions, reducing soil erosion, and ensuring the availability of various limiting nutrients (Christopher & Lal, 2007; Gould et al., 2016; Kirkby et al., 2011; Wick et al., 2017). We suspect that integrating a cocktail of root traits (morphology, quality, functionality, life cycle, among others) may help to foster these goals of improved soil health and resilience (McCormack et al., 2015) of the multifunctional Andean agroecosystem.

CONCLUSION

Findings from this study show that the association of annual and perennial forage grasses and legumes generally increases shoot and root biomass production, as well as total N uptake relative to the same species planted in monoculture, and that this trend is consistent across two distinct soil types. The success of these mixtures is likely driven by facilitation and complementarity mechanisms, and while the legume present seems to be an important factor in determining LER, it is noteworthy that the changes in LER appear to be more driven by growth of the associated grasses. Annual species tended to be the most productive in the short timeframe considered here, but establishment of perennials mixed with annual species are important to consider to ensure the continued productivity and multifunctionality of improved fallows beyond the first year. Species mixtures with perennials are likely to increase provision of quality forage and organic inputs (above and belowground) that will best support the restoration of soil health and overall agroecosystem productivity

TABLES

Table 1. Physical and chemical properties of soils used in the experiment, collected from two localities in Peru (Castillapata, Huancavelica Region and Quilcas, Junín Region)

Variable	Quilcas	Castillapata
Sand	41	63
Silt	30	28
Clay	29	9
Texture	Clay Loam	Sandy Loam
N (g kg^{-1})	1.3	5.1
Avail. P (mg kg^{-1})	14	31
Act. C (mg kg^{-1})	683.6	1229.0
OM (g kg^{-1})	20.6	80.6
K^{+} (meq $100g^{-1}$)	0.22	0.146
Ca^{+2} (meq $100g^{-1}$)	2.31	6.29
Mg^{+2} (meq $100g^{-1}$)	0.552	0.199
pH	5.64	5.89

Based on results of soil baseline analysis (Soil and Plant Nutrition Lab at La Molina University – Lima, Peru). Texture (Bouyoucos, 1962); total soil nitrogen (N) (Kjeldahl method); plant available phosphorus (P) (Olsen extraction); active carbon (POXC) (Weil et al., 2003); and exchangeable potassium (K^{+}) calcium (Ca^{2+}) and magnesium (Mg^{2+}) (ammonium acetate extraction), and pH (1:1 water:soil) (Estefan et al., 2013).

Table 2. Seeding rate for grass-legume bicultures and monocultures planted in November 2014, as well as the number of successful repetitions considered for statistical analyses in each treatment

Grasses	Legumes	Grasses (No. seeds pot^{-1})	Legume (No. seeds pot^{-1})	Quilcas (n)	Castillapata (n)
OATS	VETCH	1	2	4	4
RYEG	VETCH	2	2	4	4
FELO	VETCH	3	2	4	4
BROM	VETCH	3	2	4	3
ORCH	VETCH	3	2	3	4
OATS	REDC	1	2	4	3
RYEG	REDC	2	2	3	3
FELO	REDC	3	2	3	3
BROM	REDC	3	2	4	3
ORCH	REDC	3	2	3	4
OATS	MELU	1	2	1	4
RYEG	MELU	2	2	2	3
FELO	MELU	3	2	3	4
BROM	MELU	3	2	3	3
ORCH	MELU	3	2	3	4
OATS	ALFA	1	3	4	4
RYEG	ALFA	2	3	4	3
FELO	ALFA	3	3	3	3
BROM	ALFA	3	3	4	4
ORCH	ALFA	3	3	2	4
OATS		2		4	4
RYEG		4		4	4
FELO		6		4	4
BROM		6		3	4
ORCH		6		4	4
	VETCH		4	4	4
	REDC		4	4	4
	MELU		4	4	4
	ALFA		6	4	4

Table 3. Total biomass and total N content of grass-legume bicultures and monocultures grown in two soil types (see Table 1). The ANOVA results for each main factor and their respective interaction are presented at the bottom of the table, with significant values ($p<0.05$) in bold. Means and standard errors (SE) correspond to the original data (without transformation), while p-values are based on transformed data.

Treatments	Total biomass (g pot^{-1})				Total N (g pot^{-1})			
	Quilcas		Castillapata		Quilcas		Castillapata	
	mean	SE	mean	SE	mean	SE	mean	SE
OATS/VETCH [a]	28.33	3.80	13.01	1.79	0.40	0.12	0.22	0.01
RYEG/VETCH	15.58	1.41	13.45	3.22	0.16	0.01	0.20	0.04
FELO/VETCH	19.30	3.11	14.18	2.78	0.40	0.08	0.22	0.05
BROM/VETCH	14.42	1.33	7.36	3.88	0.38	0.02	0.17	0.08
ORCH/VETCH	17.15	1.22	8.81	1.14	0.32	0.07	0.23	0.03
OATS/REDC	29.76	2.47	8.02	3.45	0.25	0.02	0.09	0.03
RYEG/REDC	19.79	5.13	7.98	1.31	0.26	0.11	0.08	0.00
FELO/REDC	9.70	1.82	3.15	0.56	0.17	0.03	0.04	0.01
BROM/REDC	7.14	1.65	0.84	0.14	0.11	0.03	0.02	0.00
ORCH/REDC	7.06	2.90	1.96	0.79	0.11	0.03	0.03	0.01
OATS/MELU	21.14	N/A	6.14	2.05	0.15	N/A	0.05	0.01
RYEG/MELU	15.17	3.38	4.26	0.75	0.16	0.05	0.05	0.01
FELO/MELU	10.74	2.08	5.79	1.66	0.13	0.02	0.06	0.01
BROM/MELU	7.61	2.03	1.87	0.23	0.11	0.02	0.03	0.01
ORCH/MELU	8.94	1.49	1.91	0.19	0.13	0.01	0.03	0.01
OATS/ALFA	25.09	7.90	8.25	1.15	0.24	0.06	0.06	0.01
RYEG/ALFA	15.98	3.90	4.94	1.01	0.17	0.03	0.05	0.01
FELO/ALFA	9.01	4.33	5.80	1.14	0.16	0.08	0.05	0.01
BROM/ALFA	6.19	0.83	1.11	0.39	0.15	0.06	0.02	0.01
ORCH/ALFA	5.61	4.25	1.70	0.25	0.07	0.05	0.02	0.00
OATS	18.07	4.29	6.39	1.64	0.21	0.04	0.06	0.01
RYEG	15.09	2.93	6.13	2.18	0.15	0.03	0.06	0.02
FELO	14.90	3.91	3.81	0.98	0.35	0.14	0.03	0.01
BROM	9.41	1.09	1.14	0.41	0.13	0.02	0.02	0.01
ORCH	7.32	1.33	2.18	0.66	0.13	0.03	0.03	0.01
VETCH	10.54	3.22	9.47	0.52	0.32	0.10	0.29	0.02
REDC	2.97	0.89	1.22	0.50	0.10	0.03	0.04	0.02
MELU	0.92	0.53	0.56	0.33	0.03	0.01	0.02	0.01
ALFA	1.80	0.98	1.03	0.92	0.04	0.02	0.03	0.03
All treatments [b]								
Treatments	**<0.001**				**<0.001**			
Soil	**<0.001**				**<0.001**			
Soil*Treatment	0.103				0.191			
Contrast								
Biculture vs Monoculture	**<0.001**				**<0.001**			
Perennials [c]								
Treatments	**<0.001**				**<0.001**			
Soil	**<0.001**				**<0.001**			
Soil*Treatment	0.264				0.356			

Contrast Biculture vs Monoculture	**<0.001**	**<0.001**

[a] OATS=oats (*Avena sativa*; cv. Mantaro 15M), RYEG=ryegrass (*Lolium multiflorum*; cv. Tama), FELO= Festulolium (*Lolium x Festuca genera*), BROM=brome grass (*Bromus catharticus*), ORCH=orchardgrass (*Dactylis glomerata*), VETCH=vetch (*Vicia dasycarpa*), REDC = red clover (*Trifolium pratense*), MELU = black medic (*Medicago lupulina*), ALFA = alfalfa (*Medicago sativa*; cv. Ranger).

[b] P_values based on treatments involving all species, whether annular/perennial (grasses and legume).

[c] P_values based on treatments for perennial species only (grasses and legume).

Table 4. Production of roots and shoot biomass by treatment and soil type. The ANOVA results for each main factor and their respective interaction are presented at the bottom, with significant values in bold ($p<0.05$). Means and standard error (SE) correspond to the original data (without transformation), while p-values are based on the transformed data.

Treatments	Root dry biomass (g pot^{-1})				Shoot dry biomass (g pot^{-1})			
	Quilcas		Castillapata		Quilcas		Castillapata	
	mean	SE	mean	SE	mean	SE	mean	SE
OATS/VETCH [a]	4.67	1.36	1.40	0.21	23.66	3.18	11.61	1.59
RYEG/VETCH	4.88	0.78	3.52	0.76	10.70	0.71	9.93	2.51
FELO/VETCH	3.05	0.84	2.79	0.58	16.24	3.54	11.39	2.89
BROM/VETCH	1.00	0.10	0.67	0.28	13.42	1.26	6.69	3.60
ORCH/VETCH	4.85	0.72	1.15	0.30	12.30	0.53	7.66	1.09
OATS/REDC	4.78	0.63	0.86	0.28	24.98	2.12	7.16	3.17
RYEG/REDC	7.07	1.34	2.25	0.28	12.72	3.80	5.73	1.36
FELO/REDC	3.53	0.55	1.10	0.20	6.17	1.27	2.05	0.42
BROM/REDC	1.40	0.37	0.22	0.03	5.74	1.33	0.62	0.12
ORCH/REDC	2.69	1.15	0.82	0.39	4.36	1.77	1.14	0.43
OATS/MELU	5.83	N/A	0.78	0.25	15.31	N/A	5.36	1.91
RYEG/MELU	4.62	0.23	1.52	0.32	10.55	3.15	2.74	0.48
FELO/MELU	2.38	0.58	1.64	0.46	8.36	1.98	4.14	1.21
BROM/MELU	1.18	0.33	0.30	0.09	6.43	1.71	1.57	0.15
ORCH/MELU	3.18	1.06	0.66	0.05	5.76	0.44	1.24	0.17
OATS/ALFA	3.55	1.20	1.04	0.25	21.54	6.72	7.21	0.92
RYEG/ALFA	4.61	1.17	1.86	0.44	11.37	2.76	3.08	0.58
FELO/ALFA	3.08	1.53	2.12	0.10	5.93	2.82	3.68	1.06
BROM/ALFA	1.07	0.23	0.29	0.12	5.11	0.64	0.82	0.28
ORCH/ALFA	1.78	1.33	0.76	0.12	3.83	2.92	0.95	0.14
OATS	2.29	0.68	0.74	0.28	15.79	3.63	5.65	1.36
RYEG	6.08	1.36	2.24	0.82	9.01	1.58	3.89	1.36
FELO	4.80	1.13	1.48	0.39	10.1	2.87	2.33	0.68
BROM	1.35	0.18	0.22	0.07	8.06	1.27	0.92	0.35
ORCH	2.88	0.64	0.85	0.33	4.44	0.83	1.33	0.35
VETCH	0.74	0.11	0.83	0.20	9.80	3.13	8.63	0.44
REDC	0.95	0.30	0.50	0.22	2.02	0.64	0.72	0.28
MELU	0.20	0.08	0.08	0.03	0.73	0.46	0.49	0.3
ALFA	0.64	0.39	0.14	0.11	1.17	0.59	0.89	0.81
All treatments [b]								
Treatments	**<0.001**				**<0.001**			
Soil	**<0.001**				**<0.001**			
Soil*Treatment	0.018				0.120			
Contrast								
Biculture vs. monoculture	**<0.001**				**<0.001**			
Perennials [c]								
Treatments	**<0.001**				**<0.0001**			
Soil	**<0.001**				**<0.0001**			
Soil*Treatment	0.353				0.162			
Contrast								

Biculture vs. monoculture	**<0.001**	**<.0001**

[a] OATS=oats (*Avena sativa;* cv. Mantaro 15M), RYEG=ryegrass (*Lolium multiflorum;* cv. Tama), FELO= Festulolium *(Lolium* x *Festuca genera)*, BROM=brome grass (*Bromus catharticus*), ORCH=orchardgrass (*Dactylis glomerata*), VETCH=vetch (*Vicia dasycarpa)*, REDC = red clover (*Trifolium pratense)*, MELU = black medic (*Medicago lupulina)*, ALFA = alfalfa (*Medicago sativa;* cv. Ranger).[b] P_values based on treatments involving all species, whether annular/perennial (grasses and legume)[c] P_values based on treatments for perennial species only (grasses and legume).

Table 5. Land equivalent ratio (LER) for bicultures based on total biomass, roots, and shoots. ANOVA results for each major factor (grasses, legume, and soil) with their corresponding interactions are presented at the bottom, with significant values in bold (p-value<0.05). Mean LER values are presented with their corresponding standard errors (SE) to the right of each mean.

Treatments	Total LER				Root LER				Shoot LER			
	Quilcas		Castillapata		Quilcas		Castillapata		Quilcas		Castillapata	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
OATS/VETCH [a]	1.91	0.31	1.74	0.32	2.29	0.65	1.80	0.30	1.85	0.30	1.74	0.32
RYEG/VETCH	1.18	0.05	2.03	0.49	1.00	0.08	1.66	0.33	1.27	0.06	2.18	0.55
FELO/VETCH	1.89	0.37	2.75	0.53	1.18	0.10	2.10	0.43	2.04	0.46	3.05	0.79
BROM/VETCH	1.95	0.16	1.55	0.33	1.14	0.11	1.35	0.32	2.04	0.18	1.58	0.33
ORCH/VETCH	2.36	0.17	1.40	0.28	2.21	0.39	1.36	0.36	1.88	0.23	1.31	0.28
OATS/REDC	1.74	0.11	1.51	0.57	2.14	0.28	1.22	0.40	1.69	0.12	1.56	0.62
RYEG/REDC	1.38	0.37	1.55	0.22	1.27	0.26	1.16	0.10	1.46	0.44	1.68	0.38
FELO/REDC	1.25	0.34	1.05	0.20	1.52	0.43	0.86	0.14	1.12	0.30	0.97	0.11
BROM/REDC	1.00	0.33	0.72	0.13	1.15	0.34	0.75	0.20	0.97	0.33	0.75	0.10
ORCH/REDC	1.23	0.47	1.09	0.42	1.36	0.45	1.05	0.45	1.18	0.48	0.92	0.35
OATS/MELU	1.72	N/A	1.11	0.33	3.11	N/A	1.41	0.52	1.51	N/A	1.06	0.34
RYEG/MELU	1.09	0.16	1.07	0.19	0.84	0.03	1.15	0.13	1.26	0.28	1.06	0.23
FELO/MELU	0.78	0.12	1.64	0.42	0.54	0.12	1.42	0.21	0.89	0.19	1.87	0.52
BROM/MELU	2.57	1.62	2.24	0.51	2.28	1.21	2.51	1.04	2.65	1.73	2.22	0.42
ORCH/MELU	1.68	0.26	1.30	0.40	1.27	0.25	1.37	0.36	1.81	0.40	1.29	0.40
OATS/ALFA	1.48	0.44	1.40	0.25	1.61	0.54	1.73	0.52	1.47	0.42	1.35	0.21
RYEG/ALFA	1.23	0.16	0.83	0.17	0.89	0.13	0.90	0.20	1.46	0.20	0.81	0.15
FELO/ALFA	1.18	0.63	1.69	0.41	1.24	0.68	1.91	0.25	1.15	0.61	1.69	0.54
BROM/ALFA	0.73	0.08	1.02	0.37	0.85	0.16	1.84	0.91	0.71	0.07	0.90	0.31
ORCH/ALFA	0.84	0.54	0.91	0.17	0.67	0.44	1.58	0.54	0.94	0.61	0.76	0.12
All Mixture [b]												
Legume	**0.001**				0.118				**0.001**			
Grass	0.629				**0.049**				0.579			
Soil	0.966				0.679				0.882			
Legume*Grass	0.169				0.465				0.137			
Legume*Soil	0.898				0.057				0.968			
Grass*Soil	0.145				0.15				0.131			
Legume*Grass*Soil	0.813				0.766				0.865			
Perennial Mixture [c]												
Legume	0.069				0.572				**0.028**			
Grass	0.985				0.659				0.908			
Soil	0.623				0.192				0.918			
Legume*Grass	0.162				0.261				0.176			
Legume*Soil	0.836				0.057				0.944			
Grass*Soil	0.509				0.939				0.288			
Legume*Grass*Soil	0.789				0.805				0.757			

[a] OATS=oats (*Avena sativa;* cv. Mantaro 15M), RYEG=ryegrass (*Lolium multiflorum;* cv. Tama), FELO= Festulolium *(Lolium* x *Festuca genera)*, BROM=brome grass (*Bromus catharticus*), ORCH=orchardgrass

(*Dactylis glomerata*), VETCH=vetch (*Vicia dasycarpa)*, REDC = red clover (*Trifolium pratense)*, MELU = black medic (*Medicago lupulina)*, ALFA = alfalfa (*Medicago sativa;* cv. Ranger).

[b] P_values based on treatments involving all species, whether annular/perennial (grasses and legume)

[c] P_values based on treatments for perennial species only (grasses and legume).

FIGURES

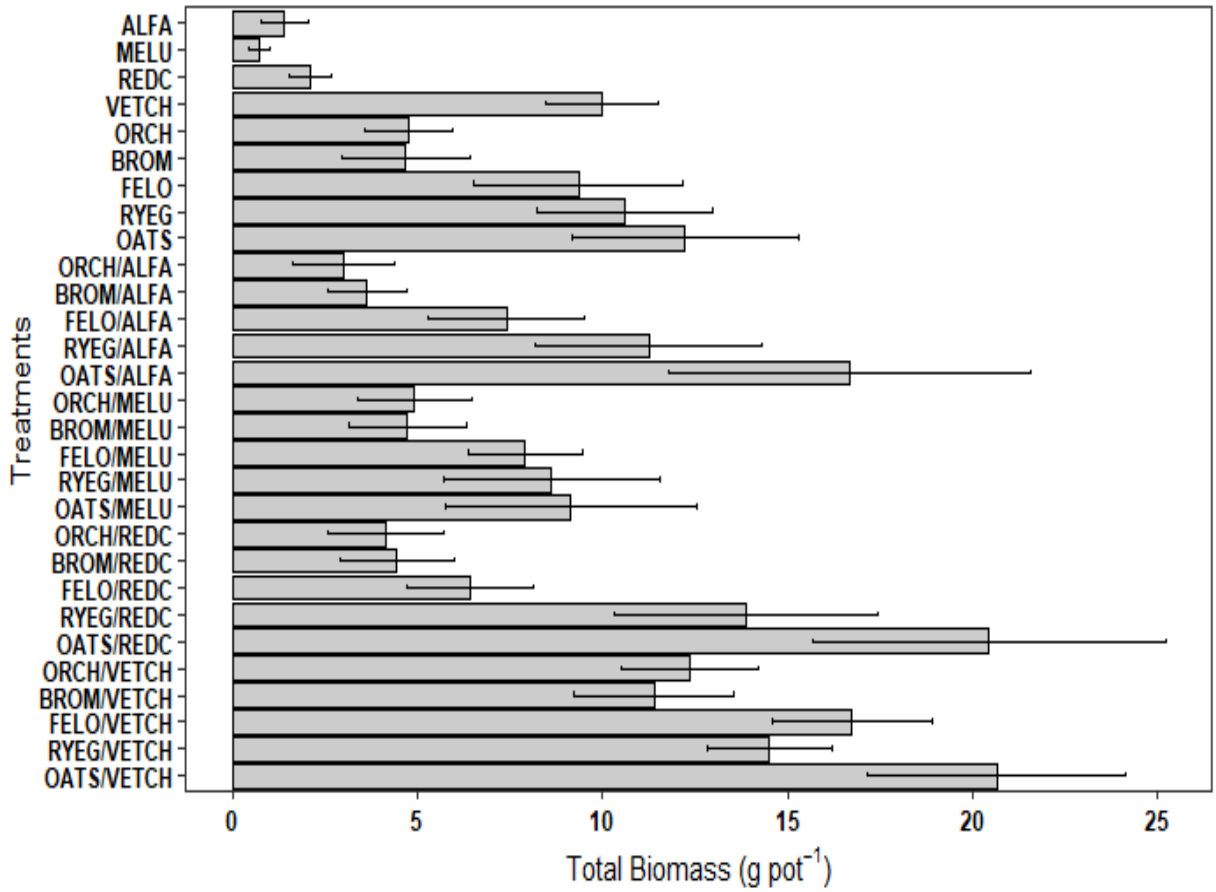

Figure 1. Mean total biomass production by treatment across soil types. Error bars present the standard error of the mean and correspond to the original data (without transformation).

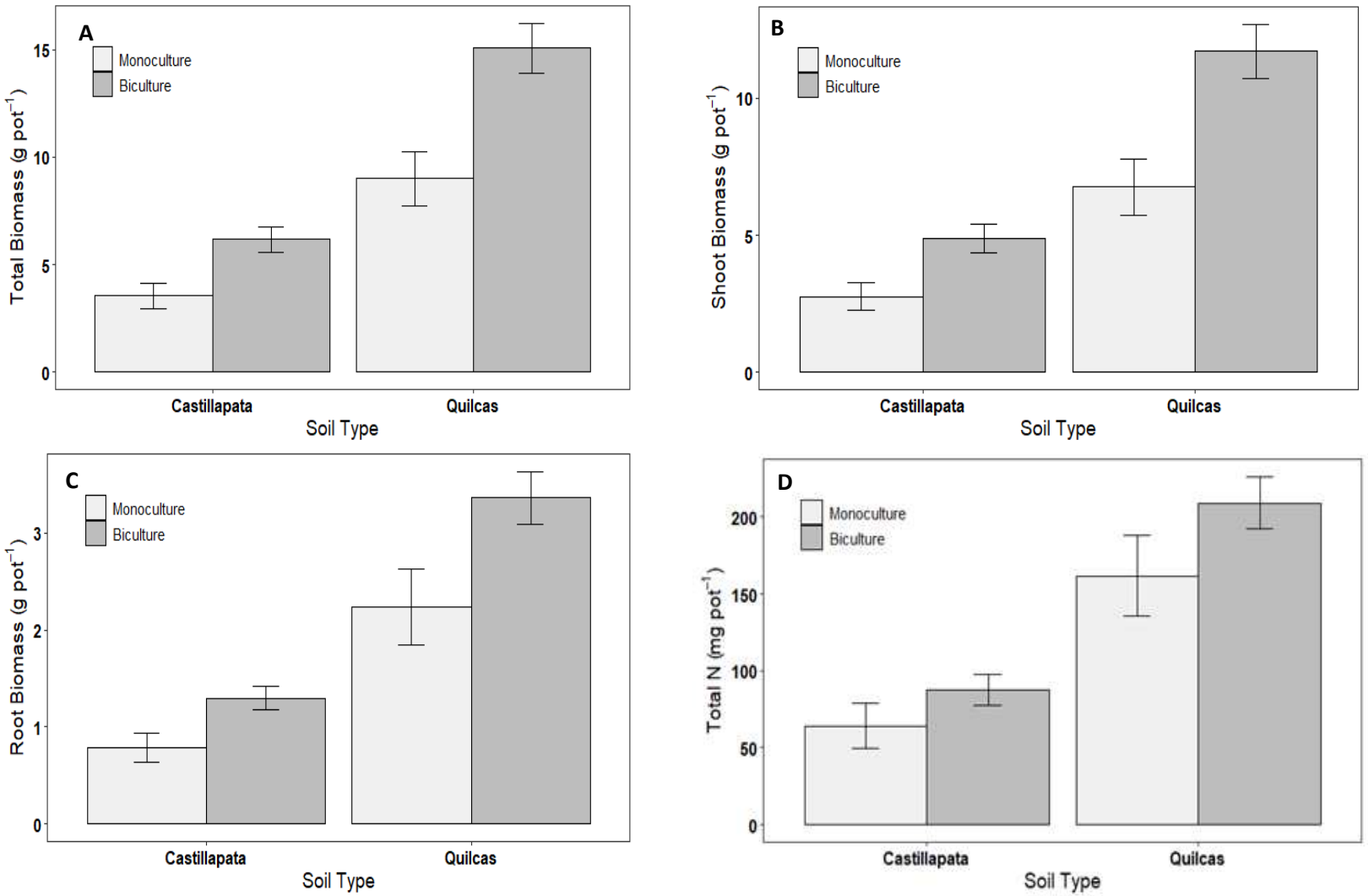

Figure 2. Mean performance of bicultures vs. monocultures (averaged across treatments) and presented separately for each soil type. Panels refer to: A. Total biomass, B. Shoot Biomass, C. Root Biomass, and D. Total N uptake. Mean and the standard error (SE) correspond to the original data (without transformation) while p-values are based on the transformed data with significant values ($p<0.05$).

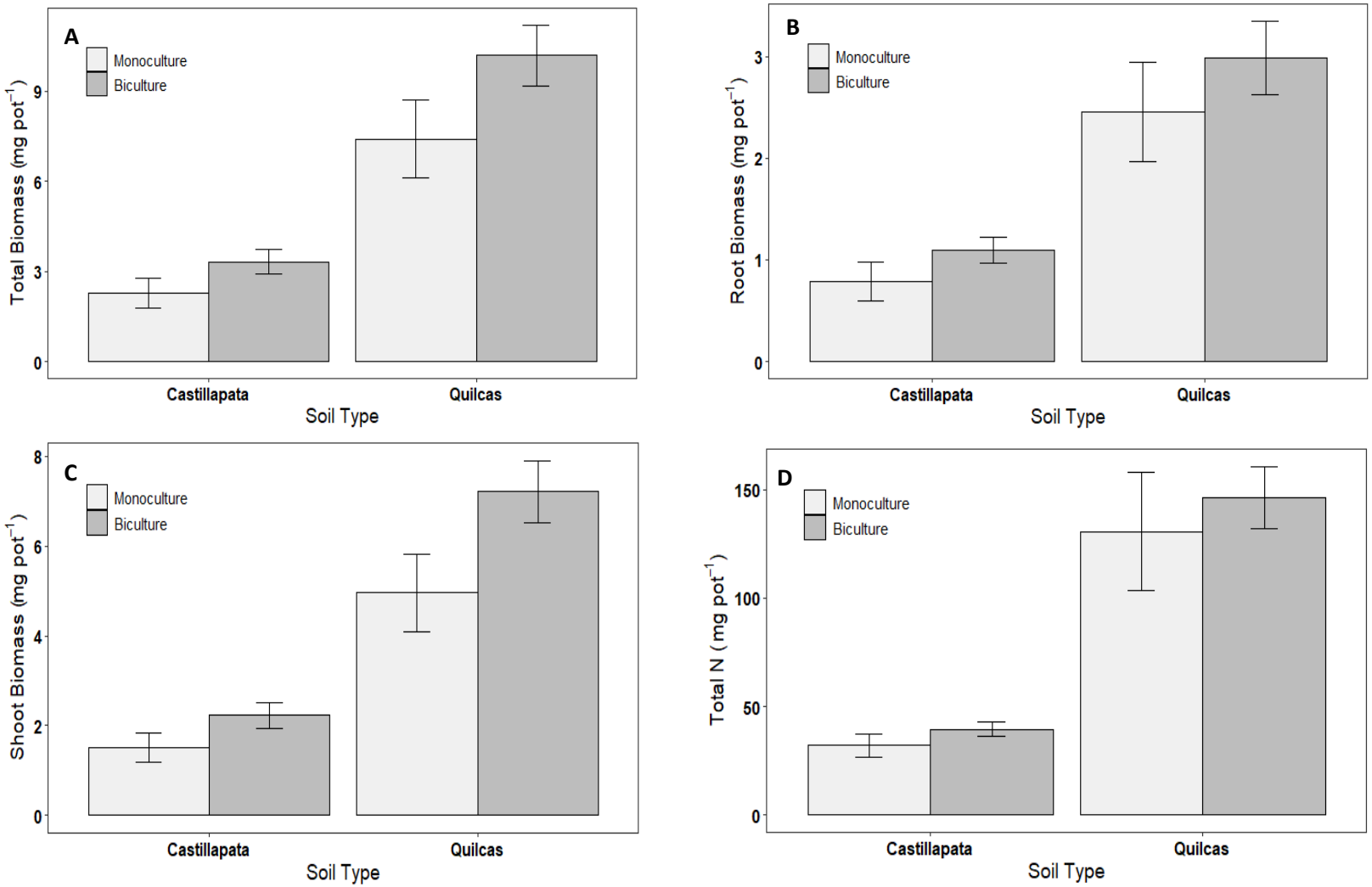

Figure 3. Mean performance values of perennial bicultures vs. monocultures (averaged across treatments with only perennial species) and presented separately for each soil type. Panels refer to: A. Total biomass, B. Shoot Biomass, C. Root Biomass, and D. Total N uptake. Mean and the standard error (SE) correspond to the original data (without transformation) while p-values are based on the transformed data with significant values ($p<0.05$).

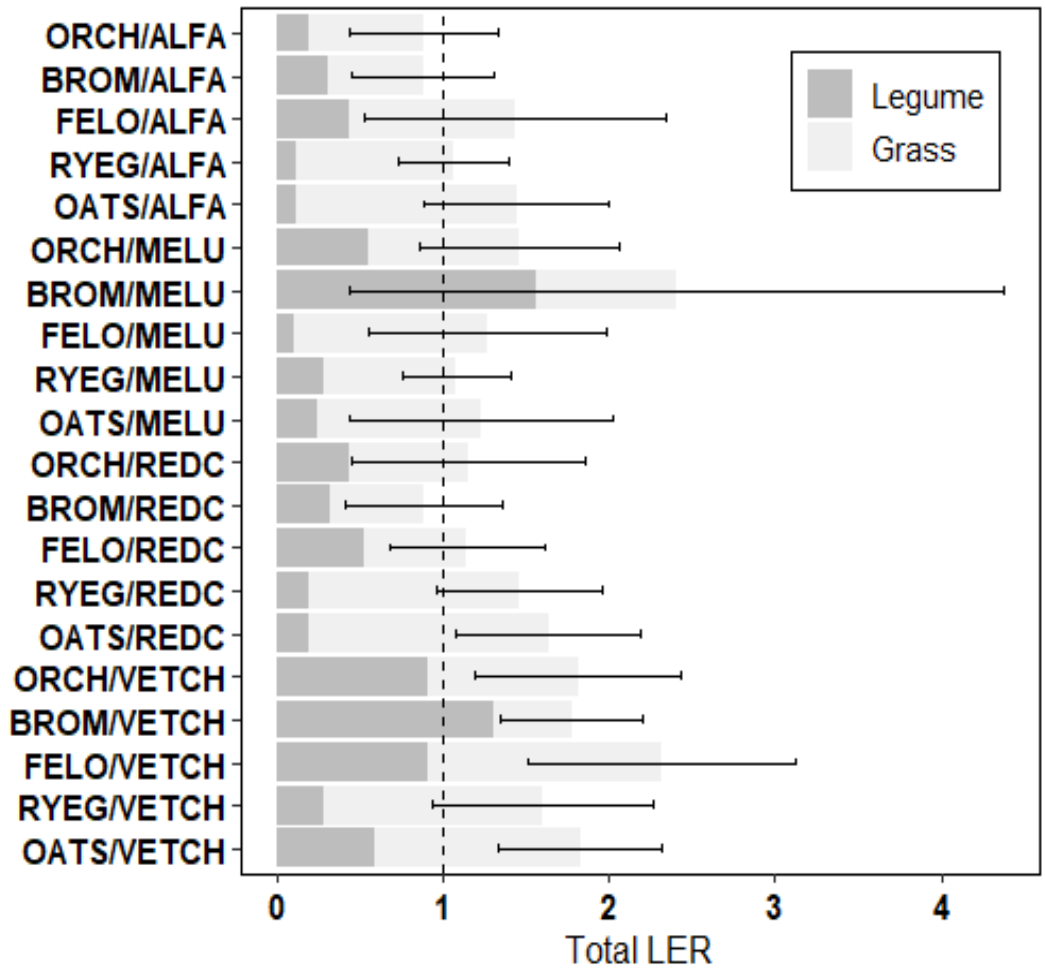

Figure 4. Land Equivalent Ratio (LER) for different treatments, corresponding to the total biomass and averaged across soil types. The bars represent 95% of the confidence interval for the estimated averages, and the values greater than 1.0 correspond to an effective biculture in relation to the monoculture.

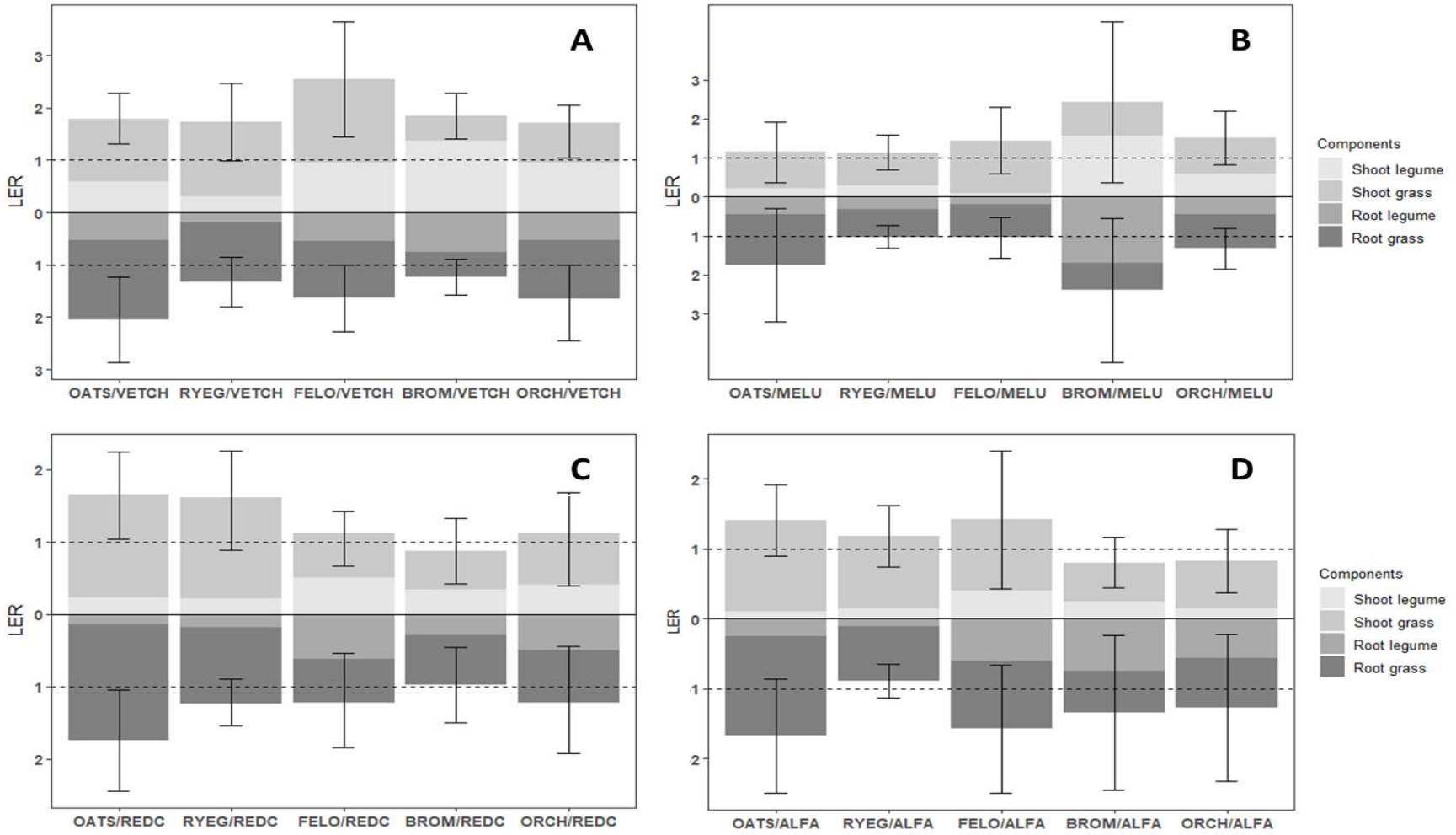

Figure 5. Land Equivalent Ratio (LER) for above and belowground biomass production of bicultures with the four legumes (A. vetch, B. black medic, C. red clover, and D. alfalfa). The bars represent the 95% confidence interval for the estimated averages, noting that all values greater than 1.0 correspond to an effective biculture in relation to the monoculture.

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APPENDICES

Table S1. Root and shoot biomass of separate grass and legume components in each treatment.

Treatments		Root dry biomass (g pot^{-1})								Shoot dry biomass (g pot^{-1})							
		Quilcas				Castillapata				Quilcas				Castillapata			
	n	Grass	SE	Legume	SE	Grass	SE	Legume	SE	Grass	SE	Legume	SE	Grass	SE	Legume	SE
OATS/VETCH	4	4.39	1.31	0.28	0.09	0.83	0.35	0.57	0.23	19.81	2.16	3.85	0.34	6.43	2.40	5.18	0.24
RYEG/VETCH	4	4.71	0.83	0.16	0.05	3.39	0.78	0.13	0.05	8.83	1.36	1.88	0.25	7.30	1.88	2.63	0.26
FELO/VETCH	4	2.58	1.05	0.48	0.22	2.40	0.53	0.39	0.08	8.44	3.67	7.81	0.77	5.54	1.55	5.85	0.36
BROM/VETCH	4	0.34	0.11	0.66	0.10	0.16	0.01	0.50	0.29	1.24	0.57	12.18	0.11	0.83	0.12	5.86	0.86
ORCH/VETCH	3	4.51	0.76	0.34	0.06	0.65	0.29	0.50	0.06	4.93	0.93	7.37	0.09	0.67	0.29	7.00	0.19
OATS/REDC	4	4.69	0.62	0.09	0.05	0.75	0.25	0.11	0.04	24.73	2.21	0.26	0.16	6.89	3.16	0.27	0.12
RYEG/REDC	3	6.95	1.30	0.12	0.05	2.12	0.27	0.13	0.02	12.60	3.75	0.13	0.11	5.47	1.36	0.25	0.03
FELO/REDC	3	2.60	1.12	0.93	0.62	0.97	0.16	0.13	0.04	4.90	2.19	1.27	0.44	1.79	0.37	0.26	0.05
BROM/REDC	4	1.05	0.31	0.36	0.29	0.12	0.06	0.10	0.03	5.04	1.06	0.70	0.28	0.39	0.19	0.23	0.10
ORCH/REDC	3	2.09	1.11	0.60	0.17	0.62	0.31	0.20	0.08	3.62	1.59	0.74	0.31	0.82	0.35	0.32	0.11
OATS/MELU	1	5.71	N/A	0.12	N/A	0.75	0.23	0.03	0.02	14.90	N/A	0.41	N/A	5.30	1.91	0.06	0.03
RYEG/MELU	2	4.61	0.25	0.02	0.01	1.48	0.34	0.04	0.02	10.49	3.21	0.07	0.12	2.54	0.53	0.20	0.15
FELO/MELU	3	2.37	0.58	0.01	0.00	1.62	0.47	0.02	0.01	8.32	1.98	0.04	0.06	4.09	1.22	0.06	0.03
BROM/MELU	3	0.86	0.50	0.32	0.29	0.17	0.03	0.13	0.08	4.95	2.71	1.48	0.64	1.04	0.11	0.53	0.18
ORCH/MELU	3	3.15	1.08	0.03	0.03	0.62	0.07	0.05	0.03	5.32	0.82	0.44	0.32	0.97	0.15	0.27	0.20
OATS/ALFA	4	3.50	1.19	0.06	0.04	0.98	0.24	0.06	0.05	21.42	6.73	0.13	0.10	7.13	0.88	0.08	0.10
RYEG/ALFA	4	4.52	1.24	0.09	0.08	1.85	0.44	0.01	0.00	11.10	2.96	0.27	0.22	3.06	0.58	0.02	0.03
FELO/ALFA	3	2.64	1.46	0.44	0.38	2.05	0.09	0.07	0.03	5.19	2.64	0.74	0.34	3.52	0.94	0.16	0.16
BROM/ALFA	4	1.01	0.25	0.07	0.04	0.10	0.05	0.19	0.13	5.01	0.66	0.10	0.09	0.46	0.22	0.36	0.16
ORCH/ALFA	2	1.74	1.35	0.05	0.01	0.64	0.06	0.11	0.07	3.70	3.00	0.13	0.12	0.81	0.10	0.14	0.07
OATS	4	2.29	0.68			0.74	0.28			15.79	3.63			5.65	1.36		
RYEG	4	6.08	1.36			2.24	0.82			9.01	1.58			3.89	1.36		
FELO	4	4.80	1.13			1.48	0.39			10.10	2.87			2.33	0.68		
BROM	3	1.35	0.18			0.22	0.07			8.06	1.27			0.92	0.35		
ORCH	4	2.88	0.64			0.85	0.33			4.44	0.83			1.33	0.35		
VETCH	4			0.74	0.11			0.83	0.20			9.80	0.73			8.63	0.08
REDC	4			0.95	0.30			0.50	0.22			2.02	0.30			0.72	0.18
MELU	4			0.20	0.08			0.08	0.03			0.73	0.30			0.49	0.26
ALFA	4			0.64	0.39			0.14	0.11			1.17	0.30			0.89	0.40